Synthesis, Characterization and Antimicrobial Evaluation of Substituted 1,2,4-Triazole Thiones Containing Pyrazole Moiety

Değiştirilmiş 1,2,4-Triazol Tiyonlarını İçeren Pirazol Parçalarının Sentezi, Karakterize Edilmesi ve Antimikrobiyal Açıdan Değerlendirilmesi

1,2,4,-Triazoller / 1,2,4-Triazoles

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Özet

Amaç:4-(3-((değiştirlmiş)-1H-pyrazol-3-yl-methylsulphonyl)-5-((3,5,6-trichloro-pyridin-2-yl-oxymeth- yl)-[1,2,4]triazol–4–yl-methyl)-morpholine (8a-g) olarak adlandırılan yeni maddeyi sentezlemek ve antimikrobiyal aktivitelerini değerlendirmek. Gereç ve Yöntem: Yeni sentezlenen ürünlerin kimyasal yapıları IR, 1H NMR, kitle spektrometrisi ve elemental analiz verileri ile aydınlatılmıştır. Staphylococcus aureus NCCS 2079, Bacillus Cereus, NCCS 2106, Escherichia coli NCCS 2065, Pseudomanas aeruginosa NCCS 2200, Aspergillus niger NCCS 1196 ve Candida albicans NCCS 2106' a yönelik anti mikrobiyal aktiviteleri disk difüzyon yöntemi ile belirlenmiş ve minimum inhibitör konsantrasyonları broth dilüsyon metodu ile gösterilmiştir. Bulgular: Ürünlerin elemental analizi göstermiştir ki deneyler yoluyla bulunan değerler teorik olarak hesaplanan değerlere çok yakındır. Her vakada kritik fonksiyone grupların IR ve 1H NMR kitle spektrometresel düzenlenmeleri belirlenmiştir. Kitle spektral fragmantasyon antimikrobiyal aktivite açısından taranan son ürünün oluşumunu kesinleştirmiştir. Her bir ürünün antibakteriyel ve antifungal aktiviteleri değerlendirilmiş ve sunulmuştur. Ürün serilerinin her bir üyesinin test edilen mikroplara karşı aktif olduğu bulunmuş, özellikle '8C' türevinin daha iyi antimikrobiyal aktivite gösterdiği izlenmiştir. Tartışma: Ürünler yeniden 1,2,4-triazollerin temel davranışını isbat eder şekilde belirgin antifungal aktivite göstermişlerdir.

Anahtar Kelimeler

1,2,4,-Triazoller; Karakterizasyon; Antimikrobiyal Aktivite; Minimum İnhibitör Konsantrasyon

Abstract

Aim: To synthesize a series of novel compounds namely, 4-(3-((substituted)-1H-pyrazol-3-yl-methylsulphonyl)-5-((3,5,6-trichloro-pyridin-2-yl-oxymeth-yl)-[1,2,4]triazol–4–yl-methyl)-morpholine (8a-g) and evaluate their antimicrobial activity. Material and Method: The chemical structures of newly synthesized compounds were elucidated by IR, ^{1}H NMR, mass spectral and elemental analysis data. Their antimicrobial activities against Staphylococus aureus NCCS 2079, Bacillus Cereus, NCCS 2106, Escherichia coli NCCS 2065, Pseudomanas aeruginos NCCS 2200, Aspergillus niger NCCS 1196 and Candida albicans NCCS 2106 were investigated by employing disk diffusion method and minimum inhibitory concentration was found out by broth dilution method. Results: Elemental analysis of the compounds indicated that the values found by the experiments were very close to theoretically calculated values. IR and ^{1}H NMR spectral assignments of critical functional groups were indicated in each case. Mass spectral fragmentation confirmed the formation of the final product to be screened for antimicrobial activity. Antibacterial and antifungal activity of each of the compounds were evaluated and presented. Each member of the series of compounds was found to be active against tested microbes in particular, the derivative '8C' was found to exhibit better antimicrobial activity. Discussion: The compounds demonstrated significant antifungal activity once again evidencing the basic trait of 1,2,4-triazoles.

Keywords

1,2,4,-Triazoles; Characterization; Antimicrobial Action; Minimum Inhibitory Concentration

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Introduction

In spite of the availability of large number of chemotherapeutic agents, there is an ever increasing demand for the new class of antibiotics due to the combination of increasing number of infectious diseases and the increasing number of multi drug resistant microbes. Though extensive research leading to the invention of new drugs is taking place, 1,2,4-triazoles nucleus has received substantial attention owing to their diverse pharmacological importance [1,2]. The versatile biological activities of triazoles are due to their ability to bind with a variety of enzymes and receptors in living system via diverse non covalent interactions [3]. In addition, triazole compounds are effective antifungal agents. Many such heavily marketed modern-day antifungal drugs containing triazole nucleus namely, fluconazole [4], voriconazole [5], itraconazole [6], ravuconazole [7] etc., are reported in the literature. The basic nitrogen of azole ring would be tightly bound to the heme iron of the fungal cytochrome P450 preventing substrate and oxygen binding, thus they act as cytochrome P450 14α-demethylase inhibitors [8]. Triazole nucleus has been a pharmacologically significant scaffold in many drug categories such as antimycobial [9], antiinflamatory [10,11], antioxidant [12], antiviral [13], antitubercular [13], anti HIV [13], antitumor [14], antimicrobial [11,15], analgesic [11], anticovasculant [16] agents, etc. These days, the fusion of medicinally important heterocyclic rings to prospective pharmaceutical candidates is a major strategy to achieve greater activity and wider medicinal spectrum. Keeping this in view, the authors herewith report the synthesis and pharmacological evaluation of 1,2,4-triazole thiones fused with pyrazole, an another medicinally important heterocyclic ring system having broad pharmacological spectrum [17-23]. A number of compounds containing diverse pharmacological importance is reported from these laboratories [24,25]

Material and Method

All Chemicals and regents were procured from Ranbaxy Laboratories Ltd, Chemical Division, India. The standard bacterial and fungal strains were procured from National Centre for Cell Sciences, Pune, India. UV-Visible spectrophotometer manufactured by Shimadzu Corporation, Japan was used for transparency measurements. Infrared spectra of compounds were recorded on Perkin-Elmer FT-IR Spectrometer. ^{1}H NMR spectra were recorded on a JOEL (300MHz) Spectrometer using TMS as an internal standard. Mass spectra were recorded on a Mass Spectrometer JOEL sx-102.

Synthetic Procedures

Synthesis of Methyl-2-(3,5,6-trichloro-pyridin-2-yloxy)-acetate (2)

Thionyl chloride (4.35 mL, 58.6 mmol, 3.0 eq) was added to a solution of 2-(3,5,6-trichloropyridin-2-yl-oxy) acetic acid (1) (5.0 g, 19.5 mmol, 1 eq.) in methanol (50 mL) at 0 °C and was heated to 80 °C for 2 hours. The reaction mixture was cooled to room temperature and concentrated under reduced pressure to remove methanol, diluted with cold water and filtered. The solid so obtained was dried under vacuum to get Methyl-2-(3,5,6-trichloro-pyridin-2-yloxy) acetate (2).

Synthesis of 2-(3,5,6-trichloro-pyridin-2-yloxy)-acetohydrazide(3).

Hydrazine hydrate (2.08 g, 41.6 mmol, 2.5 eq.) was added to a solution of Methyl-2(3,5,6-trichloro-pyridin-2-yloxy) acetate (2) (4.5 g, 16.6 mmol, 1 eq.) in ethanol (50 mL) and was refluxed for 16 hours. The reaction mixture was cooled to 0°C, filtered and the solid so obtained was washed with ethanol to get pure 2-(3,5,6-trichloro-pyridin-2-yl-oxy) aceto hydrazide (3).

Synthesis of 1-(2-(3,5,6-trichloro-pyridin-2-yloxy)-acetyl)-thio semicarbazide (4).

Potassium thiocyanate (1.08 g, 11.11 mmol, 3.0 eq.) was added to a solution of 3 (1.0 g, 3.70 mmol, 1 eq.) in acetic acid (15 mL) and was heated to 80 °C for 3 hours. The reaction mixture was cooled to room temperature, diluted with water, filtered and the solid so obtained was washed with cold water to get 1-(2-(3,5,6-trichloro-pyridin-2-yloxy)acetyl)thiosemicarbazide (4). The sample was recrystallized from a mixture of dimethyl formamide and water (1:1).

Synthesis of 5-((3,5,6-trichloro-pyridin-2-yloxy)-methyl)-2H-1,2,4-triazole-3(4H)-thione (5)

$KHCO_3$ (4.5 g, 45.7 mmol, 2 eq) dissolved in 5 mL water was added to a stirred solution of 1-(2-(3,5,6-trichloro-pyridin-2-yloxy)acetyl) thiosemi carbazide (4) (7.5 g, 22.8 mmol, 1 eq.) in a mixture of DMF : H_2O (96:24 mL) and was stirred for 16 hours. The reaction mixture was filtered, the filtrate was acidified with 2N HCl to a pH 5 and extracted with ethyl acetate. The organic layer was washed with water, brine solution, dried over anhydrous sodium sulphate and the solvent was removed under reduced pressure to get crude 5-((3,5,6-trichloro-pyridin-2-yloxy) methyl-2H-1,2,4-triazole-3(4H)-thione (5).

Synthesis of 2-((5-(1,5-diethyl)-1H-pyrazol-3-yl)-methylthio)-4H-[1,2,4]triazol-3-yl)-methoxy)-3,5,6-trichloro-pyridine (6a)

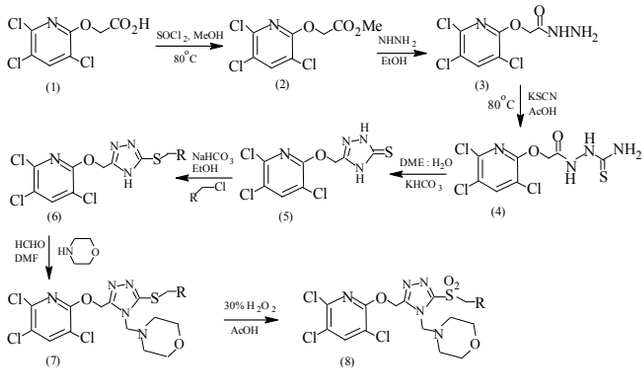
5-((3,5,6-trichloro-pyridin-2-yloxy)methyl-2H-1,2,4-triazole-3(4H)-thione (5) (200.0 mg, 0.64 mmol, 1 eq.) and 3-(chloromethyl)1,5-diethyl-1H-pyrazole (94.0 mg, 0.70 mmol, 1.1 eq.) were added to a solution of $NaHCO_3$ (108.0 mg, 1.29 mmol, 2.0 eq.) in ethanol (10 mL) and stirred for 48 hours at room temperature. The reaction mixture was concentrated under reduced pressure to remove excess ethanol, diluted with water and extracted with ethyl acetate. The organic layer was washed with water, brine solution, dried over anhydrous sodium sulphate and the solvent was removed under reduced pressure to get crude product, 2-((5-((1,5-diethyl-1H-pyrazol-3-yl) methylthio)-4H-1,2,4-triazol-3-yl)methoxy)-3,5,6-trichloro pyridine (6a). Similar procedure was followed to for the synthesis of 6b-6g.

Synthesis of 4–(3–((1,5-diethyl)–1H–pyrazol–3–yl-methylthio–5–((3,5,6–trichloro-pyridine–2–yl-oxy)-methyl)-[1,2,4]triazol–4–yl)-methyl)-morpholine (7a)

A mixture of 6a (0.1 mol), morpholine (0.15 mol) and water (20 mL) were stirred to obtain a clear solution. HCHO (0.05 mol) and DMF were added to above mixture in ice cold condition, stirred for 2 hours in an ice bath and left overnight at room temperature. White solid so obtained was isolated and recrystallized from ethanol to give 7a. The reaction procedure leading to the formation of 7a was extended for the synthesis of 7b–7g.

Synthesis of 4-(3-(1,5-diethyl-1H-pyrazol-3-yl-methylsulphonyl)-5-((3,5,6-trichloro-pyridin-2-yl-oxymethyl)-[1,2,4]triazol–4–yl-methyl)-morpholine (8a)

A solution of 0.95 g of (0.02 mol) of 7a in glacial acetic acid (50 mL) was taken in 250 mL of round bottom flask fitted with reflux condenser. The solution was heated to boil, 8 mL of 36% H_2O_2 was added and refluxed for 2 hours. The reaction mixture was cooled, filtered and the solid so obtained was recrystallized from 95% ethanol to give 8a. The reaction procedure leading to



Scheme 1. Synthesis of 4-(3-((substituted)-1H-pyrazol-3-yl-methylsulphonyl)-5-((3,5,6-trichloro-pyridin-2-yl-oxymethyl)-[1,2,4]triazol–4–yl-methyl)-morpholine (8).

Table 1. The details of substituents in various 1,2,4-triazoles synthesized

compound	R
8a	3-(chloromethyl)-1,5-dimethyl-1H-pyrazole
8b	3-(chloromethyl)-1,5-diethyl-1H-pyrazole
8c	5-tert-butyl-3-(chloromethyl)-1-methyl-1H-pyrazole
8d	3-(chloromethyl)-1-methyl-1H-pyrazole
8e	3-(chloromethyl)-1-ethyl-1H-pyrazole
8f	3-(chloromethyl)-5-ethyl-1-methyl-1H-pyrazole
8g	3-(chloromethyl)-5-isopropyl-1-methyl-1H-pyrazole

the formation of 8a was extended for the synthesis of 8b – 8g. The reaction sequence is outlined in Scheme I.

Results and Discussion

Substituted 1,2,4-triazole thiones were characterized by elemental analysis, IR and, ^{1}H NMR and mass spectral data. The details are given in the following lines.

Characterization of Methyl-2-(3,5,6-trichloro-pyridin-2-yloxy)-acetate (2)

Molecular formula, Yield, Element Calculated% (Found%): $C_8H_6Cl_3NO_3$, 86%, C 35.52 (35.12); H 2.24 (2.51); Cl 39.32 (39.53); N 5.18 (5.01), IR ν_{max} in cm^{-1} (Group): 2980 (aliphatic -CH_2); 1610 (>C=N); 1645 (ester >C=O), ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 8.81 (s, 1H, Ar-H); 5.18 (s, 2H, CO-CH_2); 3.7 (s, 3H, CO-CH_3).

Characterization of 2-(3,5,6-trichloro-pyridin-2-yloxy)-acetohydrazide (3)

Molecular formula, Yield, Element Calculated% (Found%): $C_7H_6Cl_3N_3O_2$, 66%, C 31.08 (31.61); H 2.24 (2.54); Cl 39.32 (39.11); N 15.53 (15.45), IR ν_{max} in cm^{-1} (Group): 3225 (>NH); 2980 (aliphatic -CH_2-); 1654 (>C=O of CONH), 1HNMR (300 MHz, DMSO-d_6) δ ppm: 9.2 (s, 1H, NH); 8.81 (s, 1H, Ar-H); 5.18 (s, 2H, CO-CH_2); 3.1 (s, 2H, NH_2).

Characterization of 1-(2-(3,5,6-trichloro-pyridin-2-yloxy)-acetyl)-thio semicarbazide (4)

Molecular formula, Yield, Element Calculated% (Found%): ($C_8H_7Cl_3N_4O_2S$, 59%, C 29.15 (29.67); H 2.14 (2.42); Cl 32.27 (32.04); N 17.00 (16.71); O 9.71 (9.25), IR ν_{max} in cm^{-1} (Group): 3225 (>NH); 2980 (aliphatic -CH_2-); 1654 (>C=O of CONH); 1134 (C=S), ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 9.1 (s, 1H, CONH); 8.83 (s, 1H, pyridine ring); 5.2 (s, 2H, $COCH_2$); 2.2 (s, H, NHCS); 8.32 (s, 2H, $CSNH_2$).

Characterization of 5-((3,5,6-trichloropyridin-2-yloxy)-methyl)-2H-1,2,4-triazole-3(4H)-thione (5)

Molecular formula, Yield, Element Calculated% (Found%): $C_8H_5Cl_3N_4O_2S$, 71%, C 30.84 (29.79); H 1.62 (1.82); Cl 34.14 (34.32); N 17.90 (17.61); O 5.13 (4.91), IR ν_{max} in cm$_{-1}$ (Group): 3225 (>NH); 2980 (aliphatic -CH_2-); 1654 (>C=O of CONH), 1605 (>C=N); 1134 (>C=S), ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 8.15 (s, H, HN-N- of 1,2,4-triazole-thione ring), 2.3 (s, H, -NH- of 1,2,4-triazole thione ring), 5.2 (s, 2H, -OCH_2- triazole), 8.83 (s, H, pyridine ring).

Characterization data of 2-((5-(substituted)-1H-pyrazol-3-yl)-methylthio)-4H-[1,2,4]triazol-3-yl)-methoxy)-3,5,6-trichloro-pyridine 6(a-g) [Compound: Molecular Formula, Yield (%), m.p(^{0}c), Element:Calculated% (Found%); IR ν_{max} in cm^{-1} (Group); ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm (Group)]

6a: $C_{16}H_{17}Cl_3N_6OS$, 62, 232-235, C:41.71(42.92); H:3.53(3.83); N:19.30(18.77); Cl:24.19(23.75); O:3.10(3.57); 708 (>C-S); 3229 (>NH); 1610 (>C=N-); 9.1 (s, 1H, pyridine ring); 5.35 (s, 2H, -O-CH_2-triazole); 2.35 (s, 1H, NH- of 1,2,4-triazole); 4.3 (s, 2H, S-CH_2); 6.12 (s, 1H, pyrazole =CH); 3.8 (q, 2H, >N-CH_2); 2.43 (q, 2H, C-CH_2); 1.3-1.51 (m, 6H, two -CH_3 S).

6b: $C_{17}H_{19}Cl_3N_6OS$ 60, 203-207, C:44.00(44.21); H:4.60(4.15); N:17.87(18.20); Cl:23.25(23.03); O:3.56(3.46); 715 (>C-S); 3231 (>NH); 1620 (>C=N-); 9.1 (s, 1H, Pyridine ring); 5.35 (s, 2H, -O-CH_2-Triazole); 2.35 (s, 1H, NH- of 1,2,4-Triazole); 4.3 (s, 2H, S-CH_2); 6.14 (s, 1H, pyrazole -CH); 3.92 (s, 3H, N-CH_3).

6c: C13H11Cl3N6OS 55, 223-227, C:38.49(37.59); H:2.73(2.51); N:20.7(20.67) Cl:26.22(25.91); O:3.94(4.39); 710 (>C-S); 3234 (>NH); 1615 (>C=N-); 9.1(s, 1H, pyridine ring); 5.35 (s, 2H, -O-CH_2-triazole); 2.35 (s, 1H, NH- of 1,2,4-triazole); 4.3 (s, 2H, S-CH_2); 6.12 (d, 1H, pyrazole -CH); 7.42 (d, 1H, pyrazole -CH); 3.9 (s, 3H, N-CH_3).

6d: $C_{14}H_{13}Cl_3N_6OS$ 60, 190-195, C:40.06(40.65); H:3.12(3.58); N:20.02(19.72); Cl:25.34(25.87); O:3.81(4.21); 714 (>C-S); 3236 (>NH); 1610 (>C=N-); 9.1 (s, 1H, pyridine ring); 5.35 (s, 2H, -O-CH_2-triazole); 2.35 (s, 1H, NH- of 1,2,4-triazole); 4.3 (s, 2H, S-CH_2); 6.12 (s, 1H, pyrazole -CH); 7.42 (d, 1H, pyrazole -CH); 3.79 (q, 2H, N-CH_2); 1.32 (t, 3H, -CH_3 of ethyl group).

6e: $C_{14}H_{13}Cl_3N_6OS$, 63, 210-215, C:40.06(40.45); H:3.12(3.52); N:20.02(20.43); Cl:25.34(24.83); O:3.81(4.25); 706 (>C-S); 3230 (>NH); 1608 (>C=N-); 9.1 (s, 1H, pyridine ring); 5.35 (s, 2H, -O-CH_2-triazole); 2.35 (s, 1H, -NHof 1,2,4-triazole); 4.3 (s, 2H, S-CH_2); 6.12 (s, 1H, pyrazole -CH); 3.79 (s, 3H, N-CH_3); 2.84 (s, 3H, -CH_3).

6f: $C_{15}H_{15}Cl_3N_6OS$, 63, 256-259, C:41.54(42.01); H:3.49(3.84); N:19.38(18.82); Cl:24.52(24.08); O:3.69(3.92); 715 (>C-S); 3228 (>NH); 1615 (>C=N-); 9.1 (s, 1H, pyrdine ring); 5.35 (s, 2H, -O-CH_2-triazole); 2.35 (s, 1H, NH– of 1,2,4-triazole); 4.3 (s, 2H, S-CH_2); 6.12 (s, 1H, pyrazole -CH); 3.81 (s, 3H, N-CH_3); 2.63 (q, 2H, CH_2); 1.32 (t, 3H, CH_3 of ethyl group).

6g: $C_{16}H_{17}Cl_3N_6OS$, 60, 230-234, C:42.92(42.21); H:3.83(3.59); N:18.77(19.24); Cl:23.75(22.98); O:3.57(3.89); 710 (>C-S); 3229

(>NH); 1610 (>C=N-); 9.1 (s, 1H, pyridine ring); 5.35 (s, 2H, -O-CH_2-triazole); 2.35 (s, 1H, NH- of 1,2,4-triazole); 4.3 (s, 2H, S-CH_2); 6.12 (s, 1H, pyrazole -CH); 3.82 (s, 3H, N-CH_3); 3.04 (m, 1H, $CH(CH_3)_2$); 1.32 (d, 6H, CH-$(CH_3)_2$).

Characterization data of 4–(3–((substituted)–1H–pyrazol–3–yl-methylthio–5–((3,5,6–trichloro-pyridine–2–yl-oxy)-methyl)-[1,2,4]triazol–4–yl)-methyl)-morpholine (7a-g) [Compound: Molecular Formula, Yield (%), m.p (^{0}c), Element:Calculated% (Found%) IR v_{max} in cm^{-1} (Group); 1HNMR (300 MHz, DMSO-d_6) δ ppm (Group)]

7a: $C_{21}H_{26}Cl_3N_7O_2S$, 68, 236-240, C:46.12(45.71); H:4.79(4.92); N:17.93(17.49); Cl:19.45(19.76); O:5.85(6.19); 710 (C-S); 1615 (C=N); 3040 (Aromatic pyridine ring); 9.1 (s,1H, pyridine ring); 5.35 (s, 2H, -O-CH_2-triazole); 4.3 (s, 2H, S-CH_2); 6.12 (s, 1H, pyrazole=CH); 3.8 (q, 2H, N-CH_2); 2.43 (q, 2H, C-CH_2); 1.3-1.51 (m, 6H, 2 -CH_3 groups); 4.12 (s, 2H, N-CH_2-N); 3.2(t, 4H, CH_2-O-CH_2 of morpholine ring); 2.45 (t, 4H, CH_2-N-CH_2 of morpholine ring).

7b: $C_{22}H_{28}Cl_3N_7O_2S$, 56, 196-199, C:47.11(47.29); H:5.03(4.85); N:17.48(18.88); Cl:18.96(18.22); O:5.70(6.62); 708 (C-S); 1610 (C=N); 3050 (Aromatic pyridine ring); 9.1 (s,1H, pyridine ring); 5.35 (s, 2H, -O-CH_2-triazole); 4.3 (s, 2H, S-CH_2); 6.14 (s, 1H, pyrazole CH); 3.92 (s, 3H, N-CH_3); 1.42 (s, 9H, CH_3); 4.12 (s, 2H, N-CH_2-N); 3.2 (t, 4H, CH_2-O-CH_2 of morpholine ring); 2.45 (t, 4H, CH_2-N-CH_2 of morpholine ring).

7c: $C_{18}H_{20}Cl_3N_7O_2S$, 64, 219-222, C:42.83(43.27); H:3.99(4.19); N:19.42(18.76); Cl:21.07(21.68); O:6.34(6.59); 712 (C-S); 1608 (C=N); 3044 (Aromatic pyridine ring); 9.1 (s, 1H, pyrdine ring); 5.35 (s, 2H, -O-CH_2-triazole); 4.3 (s, 2H, S-CH_2); 6.12 (d, 1H, pyrazole CH); 7.42 (d, 1H, pyrazole-CH); 3.9 (s, 3H, N-CH_3); 4.12 (s, 2H, N-CH_2-N); 3.2 (t, 4H, CH_2-O-CH_2 of morpholine ring); 2.45 (t, 4H, CH_2-N-CH_2 of morpholine ring).

7d: $C_{19}H_{22}Cl_3N_7O_2S$, 59, 203-207, C:43.98(44.25); H:4.27(4.88); N:18.90(18.64); Cl:20.50(21.04); O:6.17(6.72); 708 (C-S); 1615 (C=N); 3040 (Aromatic pyridine ring); 9.1 (s, 1H, pyridine ring); 5.35 (s, 2H,-O-CH_2-triazole); 4.3 (s, 2H, S-CH_2); 6.12 (d, 1H, pyrazole-CH); 7.42 (d, 1H, pyrazole CH); 3.79 (q, 2H, -N-CH_2); 1.32 (t, 3H, -CH_3 of ethyl group); 4.12 (s, 2H, N-CH_2-N); 3.2 (t, 4H, CH_2-O-CH_2 of morpholine ring); 2.45 (t, 4H, CH_2-N-CH_2 of morpholine ring).

7e: $C_{19}H_{22}Cl_3N_7O_2S$, 69, 215-220, C:43.98(43.81); H:4.27(4.59); N:18.90(18.46); Cl:20.50(21.09); O:6.17(6.84); 710 (C-S); 1612 (C=N); 3044 (Aromatic pyridine ring); 9.1 (s, 1H, pyridine ring); 5.35 (s, 2H, -O-CH_2-triazole); 4.3 (s, 2H, S-CH_2); 6.12 (d, 1H, pyrazole CH); 3.79 (s, 3H, N-CH_3); 2.84 (s, 3H, CH_3); 4.12 (s, 2H, N-CH_2-N); 3.21 (t, 4H, CH_2-O-CH_2 of morpholine ring); 2.45 (t, 4H, CH_2-N-CH_2 of morpholine ring).

7f: $C_{20}H_{24}Cl_3N_7O_2S$, 58, 260-264, C:45.08(45.73); H:4.54(4.75); N:18.40(18.59); Cl:19.96(20.18); O:6.00(6.75); 712 (C-S); 1615 (C=N); 3050 (Aromatic pyridine ring); 9.1(s,1H, pyridine ring); 5.35 (s, 2H, -O-CH_2-triazole); 4.3 (s, 2H, S-CH_2); 6.12 (s, 1H, pyrazole CH); 3.81 (s, 3H, N-CH_3); 2.63 (s, 2H, CH_2); 1.32(t, 3H, CH_2–CH_3); 4.12 (s, 2H, N-CH_2-N); 3.21(t, 4H, CH_2-O-CH_2 of morpholine ring); 2.45 (t, 4H, CH_2-N-CH_2 of morpholine ring).

7g: $C_{21}H_{26}Cl_3N_7O_2S$, 66, 228-235, C:46.12(46.81); H:4.79(5.18); N:17.93(17.49); Cl:19.45(20.18); O:5.85(6.31); 710 (C-S); 1608 (C=N); 3044 (Aromatic pyridine ring); 9.1 (s, 1H, pyridine ring); 5.35 (s, 2H, -O-CH_2-triazole); 4.3 (s, 2H, S-CH_2); 6.12 (d, 1H, pyrazole CH); 3.82 (s, 3H, N-CH_3); 3.04 (m, 1H, $CH(CH_3)2$); 1.32 (d, 6H, $(CH(CH_3)_2)$; 4.12 (s, 2H, N-CH_2-N); 3.21 (t, 4H, CH_2-O-CH_2 of morpholine ring); 2.45 (t, 4H, CH_2-N-CH_2 of morpholine ring).

Characterization data of 1,2,4-triazole thiones (8a-g) [Compound: Molecular Formula, Yield (%), m.p(0c), Element:Calculated% (Found%) IR v_{max} in cm^{-1} (Group); 1HNMR (300 MHz, DMSO-d_6) δ ppm (Group)]

8a: $C_{21}H_{26}Cl_3N_7O_4S$, 57, 226-230, C:43.57(43.19); H:4.53(5.05); N:16.94(16.21); Cl:18.37(17.65); O:11.06(11.92); 715 (C-S); 1615 (C=N); 1375, 1160 (O=S=O); 9.1 (s,1H, Pyridine ring); 5.35 (s, 2H, -O-CH_2-triazole) 4.67 (s, 2H, SO_2-CH_2); 6.12 (s, 1H, pyrazole=CH); 3.8 (q, 2H, N-CH_2); 2.43 (q, 2H, C-CH_2); 1.3–1.51 (m, 6H, 2 CH_3 groups); 4.12 (s, 2H, N-CH_2-N), 3.2 (t, 4H, CH_2-O-CH_2 of morpholine ring); 2.45 (t, 4H, CH_2–N–CH_2 of morpholine ring).

8b: $C_{22}H_{28}Cl_3N_7O_4S$, 59, 196-201, C:44.56 (44.21) H:4.76(4.94); N:16.54(16.71); Cl:17.94(17.62); O:10.79(11.01); 712 (C-S); 1610 (C=N); 1380, 1165 (O=S=O); 9.1 (s, 1H, pyridine ring); 5.35 (s, 2H, –O–CH_2–triazole); 4.68 (s, 2H, SO_2-CH_2); 6.14 (s, 1H, pyrazole CH); 3.92 (s, 3H, N-CH_3); 1.42 (s, 9H, CH_3); 4.12 (s, 2H, N-CH_2-N), 3.2 (t, 4H, CH_2-O-CH_2 of morpholine ring); 2.45 (t, 4H, CH_2-N-CH_2 of morpholine ring).

8c: $C_{18}H_{20}Cl_3N_7O_4S$, 63, 213-217, C:40.27(4.10); H:3.76(3.45); N:18.26(18.59); Cl:19.81(20.71); O:11.9 (12.64); 710 (C-S); 1608 (C=N); 1370, 1160 (O=S=O); 9.1 (s, 1H, pyridine ring); 5.35 (s, 2H, -O-CH_2-triazole); 4.65 (s, 2H, SO_2-CH_2); 6.12 (d, 1H, pyrazole CH); 7.42 (d, 1H, pyrazole -CH); 3.9 (s, 3H, N-CH_3); 4.12 (s, 2H, N-CH_2-N); 3.2 (t, 4H, CH_2-O-CH_2 of morpholine ring); 2.45 (t, 4H, CH_2-N-CH_2 of morpholine ring).

8d: $C_{19}H_{22}Cl_3N_7O_4S$, 54, 220-225, C:41.43(41.08); H:4.03(4.95); N:17.80(17.15); Cl:19.31(18.29); O:11.62(12.45); 708 (C-S); 1615 (C=N); 1377, 1167 (O=S=O); 9.1 (s, 1H, pyridine ring); 5.35 (s, 2H, -O-CH_2-triazole); 4.69 (s, 2H, SO_2-CH_2); 6.12 (d, 1H, pyrazole-CH); 7.42 (d, 1H, pyrazole CH); 3.79 (q, 2H, -N-CH_2); 1.32(t, 3H, -CH_3 of ethyl group); 4.12 (s, 2H, N-CH_2-N); 3.2 (t, 4H, CH_2–O–CH_2 of morpholine ring); 2.45 (t, 4H, CH_2-N-CH_2 of morpholine ring).

8e: $C_{19}H_{22}Cl_3N_7O_4S$, 59, 218-225, C:41.43 (41.22); H:4.03(4.16); N:17.80(17.74); Cl:19.31(19.57); O:11.62(11.81); 714 (C-S); 1612 (C=N); 1380, 1170 (O=S=O); 9.1 (s, 1H, pyridine ring); 5.35 (s, 2H, -O-CH_2-triazole); 4.68 (s, 2H, SO_2-CH_2); 6.12 (d, 1H, pyrazole CH); 3.79 (s, 3H, N-CH_3); 2.84 (s, 3H, CH_3); 4.12 (s, 2H, N-CH_2-N); 3.21 (t, 4H, CH_2-O-CH_2 of morpholine ring); 2.45 (t, 4H, CH_2-N-CH_2 of morpholine ring).

8f: $C_{20}H_{24}Cl_3N_7O_4S$, 68, 246-255, C:42.53(42.28) H:4.28(4.07); N:17.36(16.87); Cl:18.83(18.29); O:11.33(12.05); 710 (C-S); 1615 (C=N); 1385, 1175 (O=S=O); 9.1 (s, 1H, pyridine ring); 5.35 (s, 2H,-O-CH_2-triazole); 4.7 (s, 2H, SO_2-CH_2); 6.12 (s, 1H, pyrazole CH); 3.81 (s, 3H, N-CH_3); 2.63 (s, 2H, CH_2); 1.32 (t, 3H, CH_2-CH_3); 4.12 (s, 2H, N–CH_2–N); 3.21 (t, 4H, CH_2-O-CH_2 of morpholine ring); 2.45 (t, 4H, CH_2-N-CH_2 of morpholine ring).

8g: $C_{21}H_{26}Cl_3N_7O_4S$, 69, 225-230, C:43.57(43.71); H:4.53(4.66); N:16.94(16.25); Cl:18.37(18.88); O:11.06(11.28); 712 (C-S); 1608 (C=N); 1370, 1155 (O=S=O); 9.1 (s, 1H, pyrdine ring); 5.35 (s, 2H, -O-CH_2-triazole); 4.68 (s, 2H, SO_2–CH_2); 6.12 (d, 1H, pyrazole CH); 3.82(s, 3H, N–CH_3); 3.04 (m, 1H, $CH(CH_3)_2$); 1.32 (d, 6H, $(C(CH_3)_2)$, 4.12 (s, 2H, N–CH_2–N); 3.21 (t, 4H, CH_2-O-CH_2 of morpholine ring); 2.45 (t, 4H, CH_2-N-CH_2 of morpholine ring).

Mass spectral details

Mass spectrum of 4-(3-((1,5-diethyl)-1H-pyrazol-3-yl-methylsulphonyl)-5-((3,5,6-trichloro-pyridin-2-yl-oxymethyl)-[1,2,4]triazol–4–yl-methyl)-morpholine (8a) exhibits molecular ion (M^+) peak at m/z 551 (19.3%). The base peak was observed at m/z 354 (100%). Other prominent peaks were appeared at m/z 86 (24.7%), 160 (15.7%), 245 (27.1%), 269 (19.3%), 354 (16.6%) and 464 (27.8%).

Antimicrobial activity

The preliminary investigations pertaining to antimicrobial activity was performed by the disc diffusion method [26]. The gram positive bacteria screened were Staphylococcus aureus NCCS 2079 and Bacillus cereus NCCS 2106. The gram negative bacterial screened were Escherichia coli NCCS 265 and Pseudomonas aeruginosa NCCS2200. The fungi screened were Aspergillus niger nccs 1196 and Candida albicans NCCS 3471. Minimum inhibitory concentration was estimated by broth dilution method [27]. It can be seen from Table 2 and Table 3 that all compounds were active against tested microbes. However none of them were superior to the standards tested. It is also worth mentioning that antifungal activity of the synthesized compounds was comparable to that of standards. As mentioned supra, 1,2,4-triazoles once again proved to be better antifungal agents.

Table 2. Antimicrobial activity of 1,2,4-triazole thiones (8a-g)

Compound (40 µg/ml)	Zone inhibition (mm)					
	Staphylococus aureus NCCS 2079	Bacillus Cereus NCCS 2106	Escherichia coli NCCS 2065	Pseudomanas aeruginos NCCS 2200	Aspergillus niger NCCS 1196	Candida albicans NCCS 2106
8a	7	5	8	8	18	17
8b	5	4	5	6	15	17
8c	12	11	14	14	20	19
8d	9	7	9	8	19	18
8e	11	10	12	12	18	20
8f	8	6	8	9	19	18
8g	5	4	5	6	16	17
Cefaclor (10 µg/ml)	19	22	19	20	--	--
Ketoconazole (25 µg/ml)	--	--	--	--	22	25

Table 3. Minimum Inhibitory concentration of 1,2,4-triazole thiones (8a-g)

Compounds	Minimum inhibitory concentration (µg/ml)					
	Staphylococus aureus NCCS 2079	Bacillus Cereus NCCS 2106	Escherichia coli NCCS 2065	Pseudomanas aeruginos NCCS 2200	Aspergillus niger NCCS 1196	Candida albicans NCCS 2106
8a	19.42	21.95	18.96	19.44	6.82	7.38
8b	22.52	24.58	21.52	20.52	7.12	6.98
8c	12.56	13.88	11.28	12.52	5.28	5.82
8d	17.64	20.24	18.44	19.82	6.48	6.62
8e	15.28	16.18	13.26	14.48	6.46	6.08
8f	17.58	22.26	18.22	18.16	6.18	6.14
8g	22.58	24.62	22.52	21.52	7.08	6.58
Cefaclor	2	4	3	3	------	------
ketoconazole	-----	-----	-----	-----	0.75	0.4

Conclusion

It can be concluded that all the seven compounds synthesized namely, 4-(3-((substituted)-1H-pyrazol-3-yl-methylsulphonyl)-5-((3,5,6-trichloro-pyridin-2-yl-oxymethyl)-[1,2,4]triazol–4–yl-methyl)-morpholines (8a-g) demonstrated antibacterial and antifungal activity. Among the various 1,2,4-triazole thiones studied, 4-(3-((1-methyl)-1H-pyrazol-3-yl-methylsulphonyl)-5-((3,5,6-trichloro-pyridin-2-yl-oxymethyl)-[1,2,4]triazol–4–yl-methyl)-morpholine (8C) was found to exhibit better antibacterial and antifungal activity than the other compounds of the series.

Competing interests

Authors declare that there are competing interests.

References

1. Katritzky AR, Ramsden CA, Scriven EFV, Taylor RJK. Comprehensive Heterocyclic Chemistry III, Vol. 5. New York: Elsevier Ltd.; 2008.p.227.
2. Sugane T, Tobe T, Hamaguchi W, Shimada I, Maeno K, Miyata J, et al. Atropisomeric 4-Phenyl-4H-1,2,4-triazoles as Selective Glycine Transporter 1 Inhibitors. J Med Chem 2013 25;56(14):5744-56.
3. Zhou CH, Wang Y. Recent researches in triazole compounds as medicinal drugs. Curr Med Chem 2012;19:239-80.
4. Prasad DJ, Ashok M, Karegoudar P, Poojary B, Holla, BS, Kumari, NS. Synthesis and antimicrobial activities of some new triazolothiadiazoles bearing 4-methylthiobenzyl moiety. Eur J Med Chem 2009;44:551–7.
5. Walczak K, Gondela A, Suwiński J. Synthesis and anti-tuberculosis activity of N-aryl-C-nitroazoles. Eur J Med Chem 2004;39:849–53.
6. Turan-Zitouni G, Kaplancikli Z. A, Yildiz MT, Chevallet P, Kaya D. Synthesis and antimicrobial activity of 4-phenyl/cyclohexyl-5-(1-phenoxyethyl)-3-[N-(2-thiazolyl) acetamido]thio-4H-1,2,4-triazole derivatives. Eur J Med Chem 2005;40:607–13.
7. Holla BS, Veerendra B, Shivananda MK, Poojary B. Synthesis characterization and anticancer activity studies on some Mannich bases derived from 1,2,4-triazoles. Eur J Med Chem 2003;38:759–67.
8. Groll AH, Piscitelli, SC, Walsh TJ. Clinical pharmacology of systemic antifungal agents: a comprehensive review of agents in clinical use, current investigational compounds and putative targets for antifungal drug development. Adv Pharmacol (San Diego, CA, U. S.) 1998;44:343–500.
9. Klimesova V, Zahajska L, Waisser K, Kaustova J, Mollmann U. Synthesis and antimycobacterial activity of 1,2,4-triazole-3-benzylsulfanyl derivatives. IL. Farmaco 2004;59:279-88.
10. Mohammad A, Sadique AJ, Harish K. synthesis of some newer analogues of 4-hydroxyphenyl acetic acid as potent anti inflammatory agents. Journal of the Chinese Chemical Society 2008;55:201-8.
11. Alam MM, Nazreen S, Haider S, Shafi S, Yar MS, Hamid H, Alam MS, Synthesis of some new s-alkylated 1,2,4-triazoles, their mannich bases and their biological activities. Arch Pharm (Weinheim) 2012;345:203-14.
12. Sultan B, Evin K, Tulay C, Hatice O, Synthesis and antioxidant and antimicrobial evaluation of novel 4-substituted-1H-1,2,4-triazole derivatives. Turk J Chem 2012;36:867-84.
13. Ilkay K, Esra T. Synthesis of some novel thiourea derivatives obtained from 5-[(4-aminophenoxy)methyl]-4-alkyl/aryl-2,4-dihydro-3H- 1,2,4- triazole-3- thiones and evaluation as antiviral/anti-HIV and anti tuberculosis agents. European Journal of Medicinal Chemistry 2008;43:381-92.
14. Bhat KS, Poojary B, Prasad DJ, Naik P, Holla BS. Synthesis and antitumor activity studies of some new fused 1,2,4-triazole derivatives carrying 2,4-dichloro-5-fluorophenylmoiety. Eur J Med Chem 2009;44:5066–70.
15. Bayrak H, Demirbas A, Karaoglu SA, Demirbas N. Synthesis of some new 1,2,4-triazoles, their Mannich and Schiff bases and evaluation of their antimicrobial activities. Eur J Med Chem 2009;44:1057–66.
16. Siddiqui N, Ahsan W. Triazole incorporated thiazoles as a new class of anticonvulsants: Design, synthesis and in vivo screening. European Journal of Medicinal Chemistry 2010;45:1536–43.
17. Chauhana A, Sharma PK, Kaushik N, Kumar N. Synthesis of novel pyrazole analogues as efficacious antimicrobi al agents. Int J Pharm Pharm Sci 2011;5:166-76.
18. Anzaldi M, Maccio C, Mazzei M, Bertolotto M, Ottonello L, Dallegri F, Balbi A. Antiproliferative and proapoptotic

activities of a new class of pyrazole derivatives in HL-60 cells. Chem. Biodivers 2009;6:1674–84.
19. Bekhit AA, Ashour HMA, Bekhit AEDA, Abdel-Rahman HM, Bekhit SA. Synthesis of some pyrazolyl benzenesulfonamide derivatives as dual anti-inflammatory antimicrobial agents. J Enzym Inhib Med Chem 2009;24:296-309.
20. Bonesi M, Loizzo MR, Statti GA, Michel S, Tillequin F, Menichini. The synthesis and ACE inhibitory activity of chalcones and theirpyrazole derivatives. Bioorg Med Chem Lett 2010;20:1990–3.
21. Ouyang G, Chen Z, Cai XJ, Song BA, Bhadury PS, Yang S, et al. Synthesis and antiviral activity of novel pyrazole derivatives containing oxime esters group. Bioorgan Med Chem 2008;16:9699–707.
22. Riyadh SM, Farghaly TA, Abdallah MA, Abdalla MM, El-Aziz MRA. New pyrazolesincorporating pyrazolylpyrazole moiety: Synthesis, anti-HCV and antitumor activity. Eur J Med Chem 2010;45:1042–50.
23. Kasabe AJ, Kasabe PJ. Synthesis, anti tubercular and analgesic activity evaluation of new pyrazoline Derivatives. Int J Pharm Pharm Sci 2010;l2:132-5.
24. Venkata Narayana Rao D, Raghavendra Guru Prasad A, Spoorthy YN, Pariplavi M, Ravindranath LK. Synthesis, characterization and biological studies of substituted quinozoline-4-(3H)-one containing diazepine moiety. Annales Pharmaceutiques Françaises 2014;72:51-8.
25. Sreedevi M, Raghavendra Guru Prasad A, Spoorthy YN, Ravindranath LK, Synthesis and antimicrobial evaluation of certain novel thiazoles. Advanced Pharmaceutical Bulletin 2013;3:227-30.
26. Arthington-Skaggs BA, Motley M, Warnock DW, Morrison CJ. Comparative evaluation of PASCO and National Committee for Clinical Laboratory Standards M27-A broth micro dilution methods for antifungal drug susceptibility testing of yeasts. J Clin Microbiol 2000;38:2254-60.
27. Wiegand I, Hilpert K, Hancock RE. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc 2008;3:163-75.

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